Chapter

# Biotisation of Vegetables

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## Abstract

The research proposes a biofertiliser from mycorrhiza and rhizobium evaluating its antagonistic capacity and biotisation in the cultivation of vegetables with a DCA, the sample considers the potato, pea, and barley in the Huasahuasi Peruvian District, with nine treatments in three formulas, considering a control group without inoculation and two repetitions. As a result, the optimal formula is obtained with 300 g of mycorrhizal and rhizobium strains + 500 g of black soil + 200 g of potato peel crust, which has an effective antagonistic capacity of 100% in pea cultivation, 90% in the barley, and 85% in the potato, besides that it achieves a biotisation in the cultivation of peas of 95%, in the barley 100% and in the potato 90%.

**Keywords:** biotisation, biofertiliser, mycorrhiza, rhizobium, vegetables

## 1. Introduction

Latin American soils have low yields, chemical fertilisers are expensive, there are phytosanitary problems, soil deterioration and nitrogen deficiency in agricultural land, and at the international level, there is a search for ways to stop soil erosion, which is of great importance for the biological diversity of vegetation and fauna (**Figure 1**) [1].

One emblematic case is camu camu, which decreased to 1.5–2.5 t/ha during 2017 due to the reduction of N, K, and Mg in the soil. For this reason, it was proposed to increase production levels with biofertilizers by using cow manure, chicken manure, island guano, and river sediment.

The use of biofertilisers promotes insect repellency, increases resistance to pest and pathogen attacks through their odour (**Figure 2**) [2].

Climate change challenges agriculture, and variations in production and costs directly affect farmers [3]. In other countries, there are no soil quality problems, but pesticide residuals in products, such as tomatoes and cape gooseberries, with up to 10 pesticides found in the fruit and on the skin in concentrations of more than 0.002 ppm, toxic compounds, such as sulfotep, phorate, heptachlor, aldrin, endosulfan sulphate and I, making export impossible due to the minimum sanitary quality requirements [4].

In response, work is being done to raise awareness, proposing other forms of energy use such as alternative energies [5] and the use of arbuscular mycorrhizal fungi (AMF) together with Twin-N as biofertilisers in potato cultivation, which would completely replace the use of chemical fertilisers with a yield per hectare of

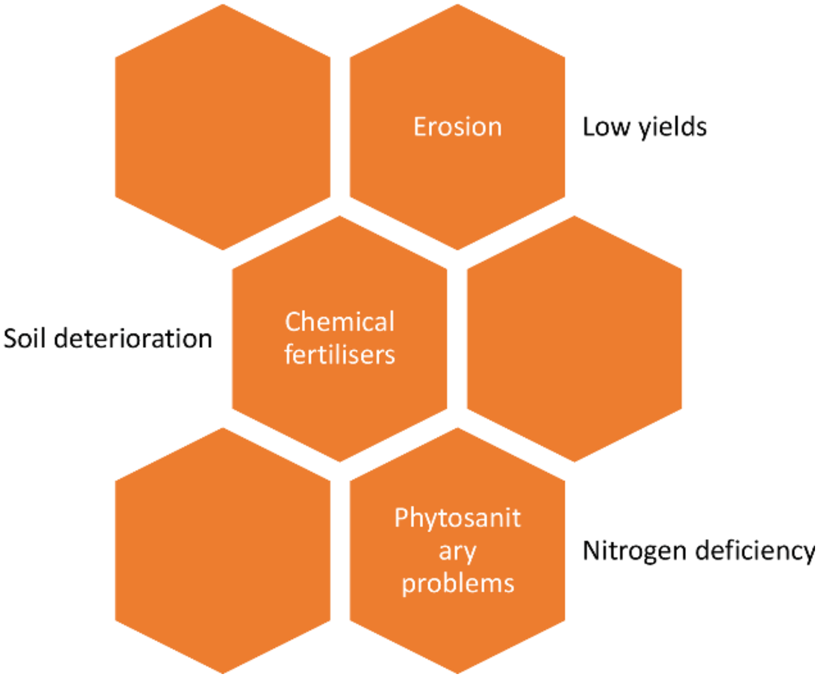

**Figure 1.**
*Soil problems.*

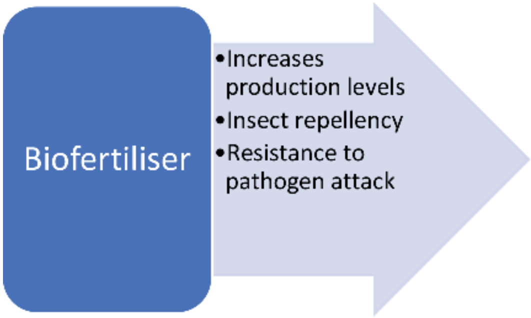

**Figure 2.**
*Benefit of biofertiliser.*

more than 116% compared to traditional fertilisation and a mostly healthy harvest of tubers with 1–10% skin lesions [6], moulds and beneficial bacteria to induce nodulation, inhibit the development of pathogenic microorganisms, fix nitrogen and other nutrients in plants, has been studied as an option for potential impact.

Mycorrhizae cover 95% of the requirements in the production of walnuts, being the production needs of 30% of nitrogen and 50% of potassium and phosphorus, the costs were 40.8% of the income from sales [7], there are studies whereby providing rhizobia a good quantity of nodules was obtained with very low weights with respect to the optimum values, but this is remedied after inoculating B. japonicum and Nod factors, offering a biotechnological alternative of acceptable yield [8].

But the antagonistic activity is another important factor, a study measures Trichoderma harzianum strains against Rhizotonia spp., Nakatea sigmoide, and Sclerotum folfsii, making T. harzianum superior in antagonism and antiparasitic activity against Garrido [9].

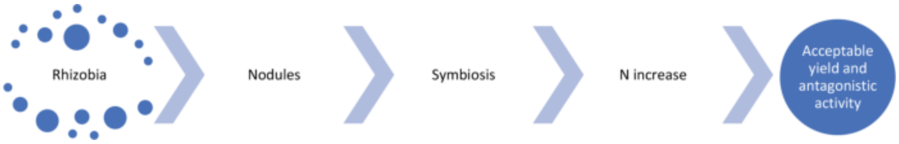

**Figure 3.**
*Rhizobium action.*

Work was carried out on wheat grains, obtaining an increase in Nitrogen (2 to 15 N/ha) and dry matter absorbed of 20–40% of that applied biofertilisers improve nutrient absorption [10].

arbuscular mycorrhizal fungi, hydrogen sulphate, and Mucoromycotina fungi are studied, which colonise 78.1% of the species, of which only 56.2% are considered to be mycorrhizal [11].

When inoculating native rhizobia on peas (Pisum sativum), 40% of the crops show the formation of nodules in symbiosis, but only 10% show their efficiency in terms of nodulation percentage and speed (**Figure 3**) [12].

Organic fertilisers in sunflower give the highest availability of nutrients, improve the weather and conditions suitable for this crop, increase the achene protein (APC), and highlight the need for water supply and sunshine on the performance of the plant development. The benefits of biofertilisation are an increase in available N which increases soil microbial activity, increases P and K content, dry matter and protein yield, the biofertiliser that obtained the highest rates of 48% oil and 14% protein is goat manure [13].

Biofertilisers were found to increase P, Ca, and Mg values but were not very effective in coffee plantations, well conventional planting systems had no differences with respect to plagiotropic branches as well as fertiliser application and type of planting [14]. Similarly, with the addition of BMV-biofertiliser, the increase in N fell and the contents of Cu and Fe decreased linearly with the increase in biofertiliser. The loss of volatile N is indicated by the alkalinity and aggregation of Ca and Mg in the oil [15].

A biofertiliser obtained by anaerobic digestion of cassava effluent was applied to the development of Crambe plants, the results indicated that the higher the percentage of biofertiliser, the oil values obtained were lower than those of the control, even that the minimum value was achieved with the highest inoculation, in addition to potassium deficiency results in decreased productivity in Crambe grains [16]. Another case is the application of cattle manure biofertiliser to strawberry plants, where it was found that production was greater than 1,250 ml/plant/week in a protected environment and sprayed with cold water and white soil, obtaining the largest fruit size in diameter and length, but with less soluble solids content (Brix) than those grown in the environment in full sun [17].

Adding saltwater to soybean reduces photosynthesis, stomatal conductance and transpiration, with low intensity when inoculated with aerobically fermented bovine biofertiliser [18], demonstrating a plant protection mechanism. When evaluating the ectomycorrhizal fungus of pine, the accumulation of heavy metals in the roots of plants with ectomycorrhizal fungi was noted, which, contrary to expectations, had fewer shoots with this type of fungi, there was no difference with the control with respect to the rhizosphere, but there was a predominance of acidobacteria, actinobacteria, and proteobacteria [19].

This study analyses mycorrhiza strains isolated from pine fungus and rhizobium isolated from pea root, thus promoting their use as biofertiliser and taking advantage of their antagonistic capacity, considering their biotisation generated from these microorganisms in plants.

It is estimated that this process contributes between 60 and 80% of biological nitrogen fixation and this symbiosis provides a considerable part of combined nutrients and nitrogen in the soil and allows plants to grow without synthetic fertilisers and without impoverishing soils [20].

## 2. Methods

The present research work was carried out in the district of San Juan de Huasahuasi, located 48 km from the district of Tarma, at an altitude of 2,751 m above sea level. The raw material consists of mycorrhiza and rhizobium isolated from pea root and pine fungus, rhizobium is diazotrophic bacteria that have the ability to fix N in plant nodules and mycorrhizae are the double absorption organs that are formed when symbiont fungi live inside healthy absorption organs (roots, rhizomes, or stems) [7, 12, 21]. Black soil and potato peel bran were used as inputs, oil and potato dextrose agar were also used, the equipment used was a microorganism incubator and an analytical balance (**Figure 4**) [22–26].

Vegetable crops were sampled—potatoes, peas, and barley in the Huasahuasi district of Tarma province.

Among the methods used were the Association of Analytical Communities *in vitro* sowing method and colony counting. Once the product was obtained, a physical-chemical characterisation was carried out, evaluating fertility, antagonistic capacity, and biotisation (**Figure 5**).

The experimental process consists of obtaining strains of microorganisms and the biofertiliser mycorrhiza and rhizobium from pea root and pine fungus through two stages:

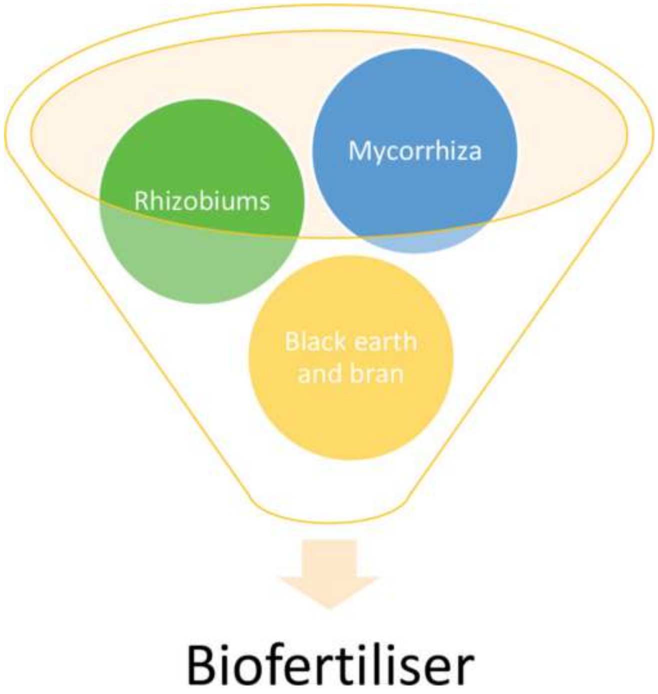

**Figure 4.**
*Elements of biofertiliser.*

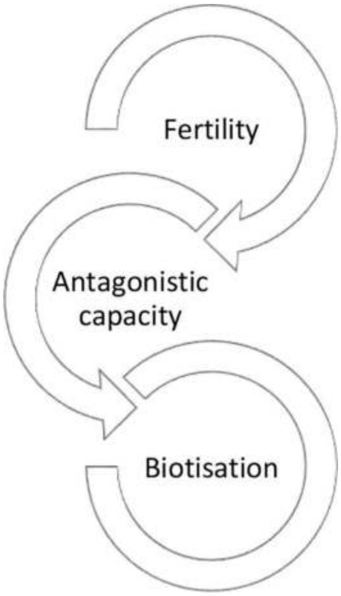

**Figure 5.**
*Characterisation of the biofertiliser.*

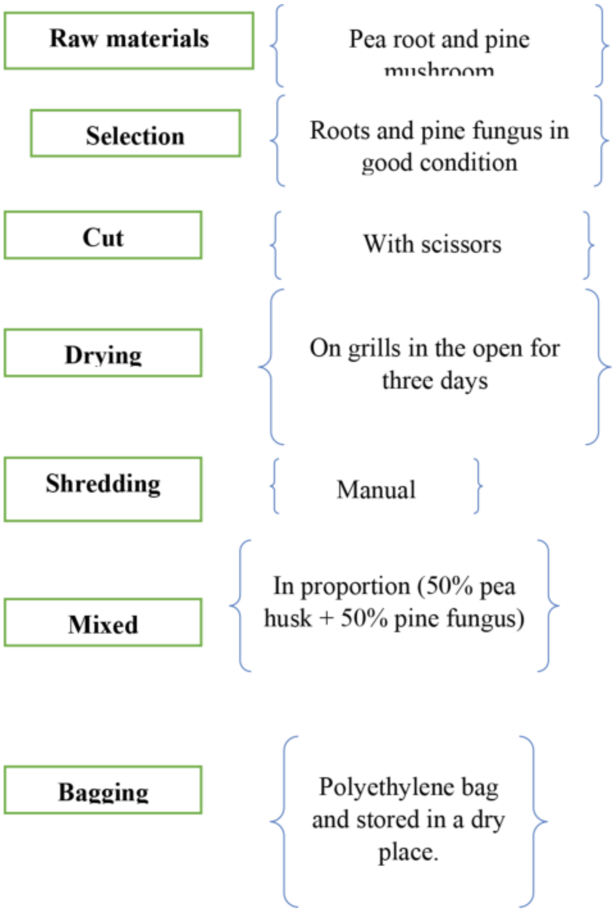

**Figure 6.**
*First stage: breeding of strains.*

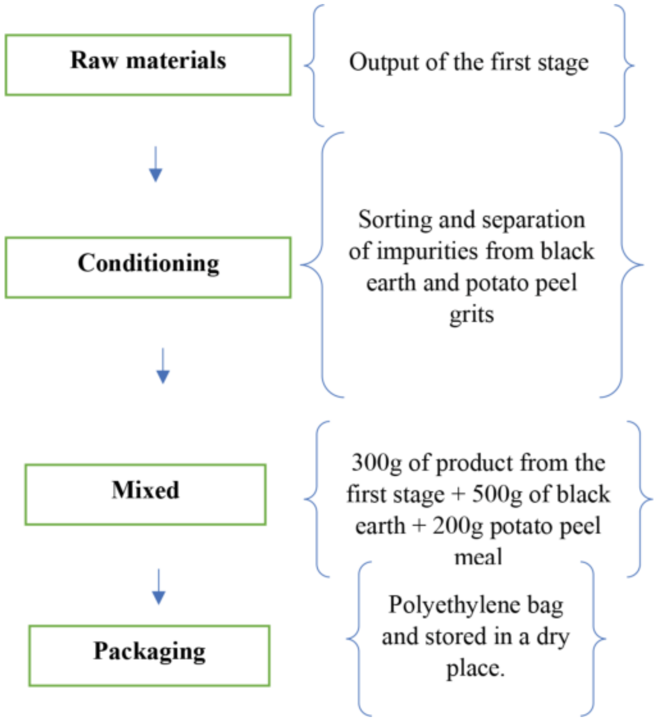

**Figure 7.**
*Second stage: obtaining the optimal biofertiliser formula.*

**Figure 8.**
*Inoculation of biofertiliser formulation to pea seed.*

The first stage consists of obtaining strains of mycorrhiza and rhizobium microorganisms, obtained from pea root and pine fungus, which is described in **Figure 6**.

The second stage consists of obtaining the optimal biofertiliser formulation of mycorrhiza and rhizobium, which is shown in **Figures 7** and **8**.

| Repetitions | Treatments | Factor A | | | |
|---|---|---|---|---|---|
| | | | 1 | 2 | 3 |
| 1 | Factor B | 1 | F1C1 | F2C1 | F3C1 |
| | | 2 | F1C2 | F2C2 | F3C2 |
| | | 3 | F1C3 | F2C3 | F3C3 |
| 2 | Factor B | 1 | F1C1 | F2C1 | F3C1 |
| | | 2 | F1C2 | F2C2 | F3C2 |
| | | 3 | F1C3 | F2C3 | F3C3 |

*F = Biofertiliser formula; F1 = 100 g of strains of microorganisms + 500 g of black soil and 200 g of potato peel bran; F2 = 200 g of strains of microorganisms + 500 g of black soil and 200 g of potato peel grist; F3 = 300 g of strains of microorganisms + 500 g of black soil + 200 g of potato peel bran; C = Vegetable cultivation (potato, pea, and barley); C1 = Potato; C2 = Peas; and C3 = Barley.*

**Table 1.**
*Relationship of biofertiliser formulation and plant cultivation.*

The statistical evaluation assessed the percentage effect of the biofertiliser in relation to its antagonistic capacity and biotisation, applying a completely randomised design (CRD) with the factorial arrangement and two replications [22], the factors were as follows:

Factor A: Inoculation of mycorrhiza and rhizobium biofertiliser formulation (F1, F2, and F3).

Factor B: Vegetable crops (potato, pea, and barley).

With a factorial arrangement of 3A × 3B = nine treatments and two replicates, the antagonistic and biotic effect of mycorrhiza and rhizobium fertiliser on vegetable crops (potato, pea and barley) is compared (**Table 1**).

## 3. Results and discussions

### 3.1 Antagonistic capacity of the biofertiliser

The analysis was applied to the treatments of the biofertiliser of mycorrhiza and rhizobium inoculating the sample with 50 g for 200 g of seed, an effective antagonistic capacity of 100% was obtained in the pea crop, 90% in barley and 85% in potato, of the biofertiliser of mycorrhiza and rhizobium with the formula F3, the biofertiliser with the formula F2 inoculated on the pea crop obtained a result of 85% effectiveness,

| Results | Crops | | | |
|---|---|---|---|---|
| **Formula** | **C1 Pea (%)** | **C2 Barley (%)** | **C3 Potato (%)** | **Effectiveness** |
| F1 | 45 | 45 | 35 | No |
| F2 | 85 | 80 | 65 | Effective |
| F3 | 100 | 90 | 85 | Effective |

*K = Ratio of strains of microorganisms 500 g mycorrhiza (pine fungus) + 500 g rhizobium (pea root).*

**Table 2.**
*Comparison of the effectiveness of antagonistic capacity.*

| Results | | Crops | | |
|---|---|---|---|---|
| **Formula** | **C1 Pea (%)** | **C2 Barley (%)** | **C3 Potato (%)** | **Effectiveness** |
| F1 | 55 | 60 | 35 | No |
| F2 | 95 | 95 | 90 | Effective |
| F3 | 95 | 100 | 90 | Effective |

**Table 3.**
*Comparison of the effectiveness of biotisation.*

80% on barley, and 65% on the potato crop, in comparison to these two formulas where the result was effective, the biofertiliser with the formula F1 the results were not very significant as the percentage of effectiveness on the pea crop was 45%, barley 45%, and potato 35%. The optimal formula of the biofertiliser of mycorrhiza and rhizobium was F3, to be used in the cultivation of vegetables, obtaining a significant result in the antagonistic capacity of the biofertiliser (**Table 2**).

### 3.2 Analysis of biotisation in vegetable crops

This analysis was carried out to verify the growth of the root system, the acclimatisation phase, and the increase in the functionality of the roots and, consequently, the nutritional and water status of the vegetable crops. The results obtained in relation to the inoculated formula and the vegetable crop used as a sample, which in this case was potato, pea, and barley, are shown in **Table 3**.

According to the results shown in **Table 3**, we can determine that the mycorrhiza and rhizobium biofertiliser has an effective effect on the biotisation of the plant crop by increasing the number of strains of microorganisms in the biofertiliser formula.

### 3.3 Balance of matter of the obtaining of the biofertiliser of Mycorrhiza and Rhizobium according to the optimal formula.

To determine the yield of the optimum formula of the mycorrhiza and Rhizobium biofertiliser, a balance of matter was carried out starting with 10 kg of pea root and 10 kg of pine fungus, the roots and fungi were selected taking into account the optimum characteristics, 16.7% was lost. The conditioning operation discards the unusable parts, stems and filaments, losing 13% during the drying operation and eliminating 55% of the water. The dry material is milled with a loss of 2%, thus obtaining a yield of 13.3 % with respect to the initial raw material (1,333 kg).

1,333 kg (50% dry pea root + 50% dry pine fungus) is mixed with 2,221 kg of black soil 0,888 kg of potato peel flour, making a total of 4,442 kg of biofertiliser for every 10 kg of fresh pea root and 10 kg of fresh pine fungus.

## 4. Economic viability

The economic feasibility assessment was carried out in each production phase and two stages.

The first stage is called obtaining the strains from the pea root and the pine fungus, both of the same proportion, these are selected, cut, crushed, and dried, in each process,

| First phase | % | Kg |
|---|---|---|
| Pea root Kg | | 10 |
| Pine mushroom Kg | | 10 |
| Selection | Loss 16.7 | 3.34 |
| Shredded | Loss 13 | 2.6 |
| Crushed | Loss 2 | 0.4 |
| Dried | Loss 55 | 11 |
| Result | Yield 13.3 | 2.66 |

**Table 4.**
*Result first phase.*

| Product | Quantity required Kg | Cost × 1 Kg | Total |
|---|---|---|---|
| Pea root Kg | 10 | 2.00 | 20.00 |
| Pine mushroom Kg | 10 | 14.00 | 140.00 |
| | | | 160.00 |

**Table 5.**
*Cost first phase.*

there are diligently measured losses between them; there is a loss of 86.7%. Based on these losses, it can be deduced that the yield in this first stage amounts to 13.3% (**Table 4**).

The second phase deals with the elaboration of the biofertiliser product from the previously obtained strains. This consists of a mixture of strains from the first phase (30%), black soil (50%), and potato peel flour (20%) (**Tables 5** and **6**).

In the beginning, 10 kg of pea root at a price of 2.00 Peruvian suns (PEN) and 10 kg of pine fungus at 14.00 PEN each Kg are used, making expenses of 20.00 and 140.00, respectively.

For the second phase, black soil is required in quantities of 4,442 kg equivalent to 50% of the total mixture of 1.5 soles, amounting to 6,663 soles and potato peel flour in quantities of 1,776 kg equivalent to 20% of 1.00 PEN, amounting to 1,776 PEN.

The labour required is three daily wages of 30.00 each making 90.00 PEN.

| | Quantity (Kg) | Cost × 1 Kg | Cost PEN | Cost GBP |
|---|---|---|---|---|
| First phase strains (30%) | 2,666 | | 160.00 | 29.79 |
| Black soil (50%) | 4,442 | 1.50 | 6,663 | 1.24 |
| potato peel flour (20%) | 1,776 | 1.00 | 1,776 | 0.33 |
| Subtotal | 8,884 | | | |
| Labour | 3 wages | 30.00 | 90.00 | 16.76 |
| | Cost 8,884 Kg | | 258,439 | 48.13 |
| | Cost Kg | | 29.09 | 5.82 |

**Table 6.**
*Cost second phase.*

With these costs and wastage, a total of 8,884 kg costs 258,439 PEN, which is 29.09 soles or 5.82 GBP per kg of biofertiliser.

The yield tests in the field show a yield of 60% when using 30 g per kg of seed potato, however, it is necessary to carry out further field tests to prove the effectiveness of each product.

Regarding the analysis of the competition, there are products, such as Trichoderma, which in its presentation of 100 g has a cost of 4.00 GBP, shipped in Ecuador in South America. Another product is the blood meal whose price per 1 Kg is 140 GBP on average and the Mycoracine that in the presentation of 500 g has a value of 543.50 GBP or the Bacillus Subtilis of 500 g at a cost of 445.00 GBP

## 5. Discussions

Antagonism is the direct inhibitory activity exerted by one microorganism on another and controls it biologically by attenuating damage to growth systems [24].

The antagonism test against soil phytopathogens was measured with respect to the fungus Fusarium Solani, the F2 and F3 formulations were effective in the antagonistic capacity against this fungus, which represents 100% of the strains, This could be due to the fact that the number of Streptomyces strains evaluated exceeded 4,000, since the inhibition zones obtained are equivalent to the average inhibition percentages obtained in the present study [23], the mechanism of antibiosis effect is presumed to be by means of inhibitory metabolites, in the same way as [24] or by repellency, as in Abanto-Rodríguez et al. [2].

Regarding the recovery of soils, the product obtained is easily used in the prevention of soil erosion [1] and can be considered as a new form of chemical energy alternative to conventional ones [5], as both mycorrhiza- and rhizobium-based products increase the amount of N in the soil as well as K and Mg, the results are similar to those obtained by Borges et al. [13], Figueiredo et al. [14], Cardoso et al. [15], and de Sousa et al. [18] for their effectiveness in biotisation.

The methodology for obtaining the biofertiliser differs from those obtained in aerobic digestion [16], in that the method proposed in this study allows the creation of a product directly proportional to its amount of addition.

Biotisation is the use of fungi and bacteria on plants that achieve acclimatisation and creation of beneficial rhizosphere [25], the effect of biotisation showed the growth of the root system, its acclimatisation and increased root functionality, comparisons show that F3 in barley is 100% effective in direct benefit to farmers [3] and similar to the findings of Flore-Córdova with the ability to replace traditional fertilisers [7] even at a lower cost as it is not necessary to inoculate boosters like Fornasero and Toniutti [8] to achieve results, These results are supported by Grageda-Cabrera et al. [10] and Lara-pérez et al. [11], but they are better than Moreno-chirinos et al. [12] as they achieve nodule formation higher than 40 % of crops, with values of 95 % in peas and even the less effective F1 formula shows 55%.

## 6. Conclusions

The biofertiliser based on mycorrhiza and rhizobium is antagonistic to the fungus Fusarium Solani, increasing its antagonistic activity by increasing the dose of these strains in the formulation.

The biofertiliser based on mycorrhiza and rhizobium is able to recover soils for the cultivation of peas, potatoes and barley.

The biofertilisation effect of the biofertiliser understudy is higher in barley (100% effective) due to its higher capacity to produce substances that stimulate plant growth.

## Conflict of interest

No conflict of interest.

## Notes/thanks/other declarations

None.

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